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TITLE: Evaluation of the Effects of Multiple Immunizations Administered in a Stressful Environment on Immunologic Function

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Background: It is hypothesized that multiple vaccinations administered simultaneously in a stressful environment may induce an exaggerated Th2 immune response and adverse health effects. Epidemiological surveys have preliminarily confirmed adverse health effects but not Th2 immune responses in multiply immunized war veterans.
Objective Hypothesis: We propose a prospective clinical trial in a military recruit population (~ 6 5 0t)o test the hypothesis that multiple, simultaneous vaccinations in a stressful environment induce an exaggerated Th2 immune response in addition to adverse Th2-associated symptoms.
Specific Aims: This study aims to compare the immune responses and health effects in recruits undergoing a multiple, simultaneous vaccination schedule with the s a l e variables in those immunized with a staggered schedule.
Study Design: A Marine recruit population with routine high levels of stress will be split into (I) multiple, simultaneous and (2) staggered vaccination groups. Cytokine and lymphocyte levels in addition to lymphocyte stimulation studies will be performed on blood samples to compare immune responses. Questionnaires, sick call databases, and comprehensive electronic military health databases will be used to compare health outcomes.
Relevance: Immunity to infectious pathogens is critical for maintaining military readiness, but the potential effects of multiple, simultaneous vaccinations are not well known. This study will contribute to existing research on the possible impact of multiple vaccinations administered under stressful conditions.

15. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)
multiple vaccination, immune response, stress, cytokine, Th2, military

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Introduction:

Recruits receive multiple vaccinations as part of their preparation for basic training because the stressful and crowded conditions provide opportunities for outbreaks of disease. In many basic training camps, the majority of vaccinations are given on the same day. It is hypothesized that the administration of multiple vaccines simultaneously negatively affects immune function and can lead to adverse health effects when combined with stressful conditions. Despite the evidence, none of the investigations performed the immunological tests necessary to determine the role of a change in immune function (cytokine levels) when multiple vaccinations are given along with stressful conditions. We proposed a prospective clinical trial with military recruits to test if multiple vaccinations given simultaneously in a stressful environment lead to Th2 cytokine imbalance and associated adverse health effects.

Body:

The purpose of this study is to determine if multiple, simultaneous vaccinations given in a stressful environment induces a Th2 cytokine shift and/or causes adverse health effects.

Participants were randomly split into two groups. The first group received the Current Schedule (CS) of vaccinations at the recruit training command (largely simultaneous), and the second group received their vaccinations by a Staggered Schedule (SS), receiving the same shots but split into three different periods throughout boot camp. Table 1 demonstrated the two schedules. Changes to immunologic levels through the three blood draws were evaluated through the use of lymphocyte stimulation studies. The ratio of Th2 to Th1-associated cytokines was evaluated for the two arms. Saliva and serum levels are also monitored for cortisol levels, as surrogates for stress levels. Also, visits to the clinic and hospital by all participants were monitored, and categorized into specific groups, i.e. respiratory, muscular, GI, and psychological illnesses. Specific tasks outlined in the Statement of Work with status of completion at the time of this reporting follow:

Task 1. Determine if multiple vaccinations administered simultaneously in a stressful environment induce a Th2 immune response or other irregularities in immune function.

a. Perform initial blood draw and analysis to determine baseline immunologic data (day 1).*

Status: Initial blood draw completed on all subjects enrolled (total n = 650).

b. Create two study populations by vaccinating half the total population according to a multiple, simultaneous (MS) vaccination schedule (day 1) and the other half according to a staggered schedule (SS) (days 1 and 35). Three hundred twenty-five individuals will be recruited for each arm of the study. Given attrition (study attrition and recruit camp attrition) of 20%, a total of approximately 260 will remain in each arm, meeting sample size calculation needs.

Status: Enrollment was completed with 324 CS participants and 331 SS participants enrolled. Attrition was 17.8%. All participants completed the follow-up portion of the study as of 19 April 2006. The last phase of continued monitoring of visits to clinics and hospitals lasted approximately for one year, per the protocol (April 2007). From this date, it takes approximately 6 months for the electronic data records to "mature", allowing final analysis of post-study health care exposures (late 2007 final analysis was initiated).

c. Perform blood draws for immunologic analyses detection of immune response (days 1, 30 and 45).

Status: Performed on all remaining participants (attrition is the result of, among other reasons, removal from training and return to home).

d. Compare cytokine profile and immune function indicator data between MS and SS groups (completed by first year of study).

Status: Lab work completed. Preliminary results below.

Task 2. Determine if the multiple vaccinations administered simultaneously in a stressful environment lead to adverse health effects that are proportional to the Th2 shifts.

a. Administer initial questionnaire to determine baseline symptomologic health data (day 1).

Status: Completed.

b. Create two study populations, MS and SS, as indicated above (days 1 and 35).

Status: Completed.

c. Perform passive surveillance of subject health through sick call databases for the duration of training to assess short-term effects (days 1-84).

Status: Completed.

d. Administer final questionnaire to determine symptomologic health changes throughout training (day 70).

Status: Completed.

e. Perform passive surveillance of subject health through comprehensive medical databases for one year after the completion of training to assess long-term effects.

Status: Completed.

f. Compare health-effect data between MS and SS groups (completed by second year of study).

Status: Completed.

Table 1

Vaccination Schedules	Day 1	Day 30	Day 45
Current Vaccination Schedule (CS)	Meningococcal MMR (live) Hepatitis A/B #1 Tetanus-diphtheria IPV Varicella #1 (live) Yellow fever (live)	Hepatitis A/B Varicella #2 (live)	
Staggered Vaccination Schedule (SS)	Meningococcal MMR (live) Varicella #1 (live)	Varicella #2 (live) Yellow Fever (live)	IPV Hepatitis A/B #1 Tetanus-diphtheria
Phlebotomy and Questionnaire Schedule (all subjects)*	Phlebotomy Questionnaire Saliva Sample	Phlebotomy Saliva Sample	Phlebotomy Questionnaire Saliva Sample

Key Research Accomplishments:

Data, via self-administered questionnaire, cytokine profile monitoring, review of sick call visits, and examination of comprehensive medical databases was collected from 655 volunteer subjects, (exceeding the original goal of 650 subjects due to # of desired participants on the final day of enrollment), through the period of one year to assess long-term effects. This extended period gives strong validity to the study conclusion.

Products:

1. The project was presented at the Navy Occupational Health and Preventive Medicine Workshop, Virginia Beach, VA, 19-23 Mar 2006. The poster won a first place ribbon. (Abstract previously provided).
2. Russell KL, Hansen C, Faix D, Blasiole D, Ryan MAK, Myers C. Vaccinations Administered in a Grouped or Staggered Schedule and Respiratory Outcomes among US Navy Recruit Trainees. X International Symposium on Respiratory Viral Infections, 28 Feb-02 March, Singapore. Accepted poster presentation. (Abstract Attached).
3. Peer-Reviewed publication in preparation.

Reportable Outcomes:

Survey results at study enrollment indicated no significant differences between treatment groups, including gender, race, history of use of tobacco and alcohol, and overall health and fitness. Significantly more health care encounters were observed in participants receiving the single grouping of vaccinations (13.1% more than staggered group, p-value 0.003), with respiratory diagnoses representing the greatest difference (24.1% more than staggered group, p-value 0.008). Additionally, analysis of time to first respiratory diagnosis via electronic health care records indicates a significantly decreased risk among trainees receiving the staggered vaccination schedule when compared with the group receiving clustered vaccinations (H.R. 0.75, 95% CI: 0.59-0.91; p-value = 0.007). Analysis of survival curves indicates the difference in rates of health care utilization becomes evident approximately 25 days after enrollment.

The immunological response to receiving several vaccinations simultaneously in a stressful environment was measured, and compared to the same effects of receiving several vaccinations, but in a staggered schedule.

Data collected supports each of the following original hypotheses:

1) Multiple, simultaneous vaccinations given in a stressful environment induces an exaggerated Th2 immune response.
2) Multiple, simultaneous vaccinations given in a stressful environment leads to adverse health effects potentially corresponding to the Th2 shifts.

Conclusions:

The comparatively small modification to the routine schedule of vaccinations evaluated in this study was associated with significant differences in the immune response induced, and significantly less respiratory disease among military recruits. This may be an acceptable adjunct to other intervention techniques in decreasing the rates of respiratory illness, and the associated morbidity, among recruits in training. Data to support the hypotheses outlined through Tasks 1 and 2 was gathered. Final peer-review and publication is pending.